Applicant: The General Hospital Corporation *et al.* BOX PCT

PCT Int'l. Appl. No: TO BE ASSIGNED Docket: 0609.444PC01

PCT Int'l. Filing Date: 12 March 1998 Atty: JAG/BEC

For: **A METHOD FOR TREATING OR PREVENTING ALZHEIMER'S DISEASE**

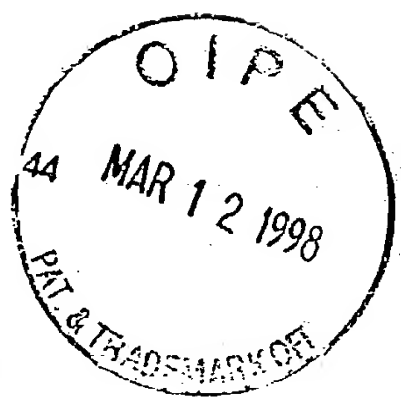
When receipt stamp is placed hereon, the USPTO acknowledges receipt of the following documents:

The International PCT Application contains:

1. PCT Transmittal Letter (1 Sheet); Fee Calculation Sheet;
2. PCT Request Form (5 Sheets);
3. Description (11 Sheets); Claims (3 Sheets); Abstract (1Sheet); Informal Drawings (0 Sheets)
4. SKG&F Check No. 21331 in the amount of $ 1,920.00.

(Due Date: 12 March 1998)

pjs



noted 3/13/98

TRANSMITTAL LETTER TO THE UNITED STATES RECEIVING OFFICE

I	12 March 1998
International Application No.	TBA
Attorney Docket No.	0609.444PC01

I. Certificate under 37 C.F.R. § 1.10 (if applicable)

Express Mail mailing number	Date of Deposit

I hereby certify that the application/correspondence attached hereto is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, DC 20231.

Signature of person mailing correspondence	Typed or printed name of person mailing correspondence

II. ☒ New International Application

TITLE	A METHOD FOR TREATING OR PREVENTING ALZHEIMER'S DISEASE	Earliest priority date (Day/Month/Year) 12 March 1997 (12.03.97)

SCREENING DISCLOSURE INFORMATION: In order to assist in screening the accompanying international application for purposes of determining whether a license for foreign transmittal should and could be granted and for other purposes, the following information is supplied. (Note: check as many boxes as apply):

A. ☐ The invention disclosed was **not** made in the United States.

B. ☐ There is no prior U.S. application relating to this invention.

C. ☒ The following prior U.S. application(s) contain subject matter which is related to the invention disclosed in the attached international application. *(NOTE: priority to these applications may or may not be claimed on form PCT/RO/101 (Request) and this listing does not constitute a claim for priority)*

application no.	60/039,607	filed on	12 March 1997 (12.03.97)

D. ☐ The present international application ☐ is identical to ☐ contains less subject matter than that found in the prior U.S. application(s) identified in paragraph C above.

E. ☒ The present international application ☒ contains additional subject matter not found in the prior U.S. application(s) identified in paragraph C above. The additional subject matter is found throughout the specification.

and ☒ **DOES NOT ALTER** ☐ **MIGHT BE CONSIDERED TO ALTER** the general nature of the invention in a manner which would require the U.S. application to have been made available for inspection by the appropriate defense agencies under 35 U.S.C. § 181 and 37 C.F.R. § 5.15.

III. ☐ A Response to an Invitation from the RO/US. *The following document(s) is(are) enclosed:*

A. ☐ A Request for An Extension of Time to File a Response

B. ☐ A Power of Attorney (General or Regular)

C. ☐ Replacement pages

pages		of the claims

D. ☐ Submission of Priority Documents

Priority document		Priority document	

E. ☐ Fees as specified on attached Fee Calculation sheet form PCT/RO/101 annex

IV. ☐ A Request for Rectification under PCT Rule 91 ☐ **A Petition** ☐ **A Sequence Listing Diskette**

V. ☐ Other (please identify):

The person signing this form is the:

- ☐ Applicant
- ☒ Attorney/Agent (Reg. No.) 29,021
- ☐ Common Representative

MICHELE A. CIMBALA Reg. No. 33851
for Jorge A. Goldstein
Typed name of signer

Michele A. Cimbala
Signature

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For Receiving Office Use Only
International Application No.
International Filing Date
Name of receiving Office and "PCT International Application"
Applicant's or agent's file reference (if desired) (12 characters maximum) 0609.444PC01

Box No. I TITLE OF INVENTION A METHOD FOR TREATING OR PREVENTING ALZHEIMER'S DISEASE

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e., country) of residence if no State of residence is indicated below.)*

THE GENERAL HOSPITAL CORPORATION
Fruit Street
Boston, Massachusetts 02114
United States of America

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e., country) of residence if no State of residence is indicated below.)*

ESMOND, Robert W.
312 Blair Court
Vienna, Virginia 22180
United States of America

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of: ☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☒ **Further applicants and/or (further) inventors are indicated on a continuation sheet.**

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

GOLDSTEIN, Jorge A.
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, N.W. - Suite 600
Washington, D.C. 20005-3934
United States of America

Telephone No.
(202) 371-2600

Facsimile No.
(202) 371-2540

Teleprinter No.
248636 SSK

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e., country) of residence if no State of residence is indicated below.)*

WANDS, Jack R.
210 Varick Road
Waban, Massachusetts 02168
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of: ☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e., country) of residence if no State of residence is indicated below.)*

de la MONTE, Suzanne
42 Middlesex Street
Cambridge, Massachusetts 02140
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of: ☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e., country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e., country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATIO. OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked):*

Regional Patent

☐ **AP ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe and any other State which is a Contracting State of the Harare Protocol and of the PCT.

☐ **EA Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT.

☒ **EP European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT.

☐ **OA OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* . . . . . .

National Patent *(if other kind of protection or treatment desired, specify on dotted line):*

☐	AL	Albania	☐	LT	Lithuania
☐	AM	Armenia	☐	LU	Luxembourg
☐	AT	Austria	☐	LV	Latvia
☐	AU	Australia	☐	MD	Republic of Moldova
☐	AZ	Azerbaijan	☐	MG	Madagascar
☐	BA	Bosnia and Herzegovina	☐	MK	The former Yugoslav Republic of Macedonia
☐	BB	Barbados			
☐	BG	Bulgaria	☐	MN	Mongolia
☐	BR	Brazil	☐	MW	Malawi
☐	BY	Belarus	☐	MX	Mexico
☒	CA	Canada	☐	NO	Norway
☐	CH and LI	Switzerland and Liechtenstein	☐	NZ	New Zealand
☒	CN	China	☐	PL	Poland
☐	CU	Cuba	☐	PT	Portugal
☐	CZ	Czech Republic	☐	RO	Romania
☐	DE	Germany	☐	RU	Russian Federation
☐	DK	Denmark	☐	SD	Sudan
☐	EE	Estonia	☐	SE	Sweden
☐	ES	Spain	☐	SG	Singapore
☐	FI	Finland	☐	SI	Slovenia
☐	GB	United Kingdom	☐	SK	Slovakia
☐	GE	Georgia	☐	SL	Sierra Leone
☐	GH	Ghana	☐	TJ	Tajikistan
☐	GM	Gambia	☐	TM	Turkmenistan
☐	GW	Guinea-Bissau	☐	TR	Turkey
☐	HU	Hungary	☐	TT	Trinidad and Tobago
☐	ID	Indonesia	☐	UA	Ukraine
☐	IL	Israel	☐	UG	Uganda
☐	IS	Iceland	☒	US	United States of America
☒	JP	Japan			
☐	KE	Kenya	☐	UZ	Uzbekistan
☐	KG	Kyrgyzstan	☐	VN	Viet Nam
☐	KP	Democratic People's Republic of Korea	☐	YU	Yugoslavia
			☐	ZW	Zimbabwe
☐	KR	Republic of Korea	Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet.		
☐	KZ	Kazakstan			
☐	LC	Saint Lucia	☐		
☐	LK	Sri Lanka	☐		
☐	LR	Liberia	☐		
☐	LS	Lesotho			

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Supplemental Box *If the Supplemental Box is not used, this sheet need not be included in the request.*

Use this box in the following cases:

1. If, in any of the Boxes, the space is insufficient to furnish all the information:	***in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient;***
in particular:	
(i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available:	*in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (i.e, country) of residence if no State of residence is indicated below;*
(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked:	*in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*
(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America:	*in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eursian, European or OAPI patent) for the purposes of which the named person is inventor;*
(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents:	*in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*
(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "Continuation" or "Continuation-in-part":	*in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*
(vi) if there are more than three earlier applications whose priority is claimed:	*in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.*
2. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty:	***in such case, write "Statement Concerning Non-Prejudicial Disclosures or Exceptions to Lack of Novelty" and furnish that statement below.***

Continuation of Box IV

STERNE, Robert Greene; KESSLER, Edward J.; FOX, Samuel L.;; CORNWELL, David K.S.; ESMOND, Robert W.; DURKIN, Tracy-Gene G.; CIMBALA, Michele A.; RAY, Michael B.; SOKOHL, Robert E.; STEFFE, Eric K.; LEE, Michael Q.

All of the above are members of the firm of:

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, N.W. - Suite 600
Washington, D.C. 20005-3934
United States of America

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1) US	12 March 1997 (12.03.97)	60/039,607	

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☒ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certifed copy of the earlier application(s) identified above as item(s): (1)

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA / US

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office): Date *(day/month/year):* Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	:	5	sheets
2. description	:	11	sheets
3. claims	:	3	sheets
4. abstract	:	1	sheets
5. drawings	:	0	sheets
Total	:	20	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):**
5. ☒ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☒ other *(specify): transmittal letter*

Figure No. none of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Michael A. Cantuchi

for Jorge A. Goldstein

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings ☐ received ☐ not received
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority specified by the applicant: ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT
FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only

International application No.

Applicant's or agent's file reference: 0609.444PC01

Date stamp of the receiving Office

Applicant The General Hospital Corporation *et al.*

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . 240.00 [T]
2. SEARCH FEE . . . . . 700.00 [S]

International search to be carried out by ISA/US

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 20 sheets.

first 30 sheets . . . . . 455.00 [b_1]

0 (remaining sheets) x 10.00 (additional amount) = 0.00 [b_2]

Add amounts entered at b_1 and b_2 and enter total at B . . . . 455.00 [B]

Designation Fee

the international application contains 5 designations

5 (number of designations payable (*maximum* 11)) x 105.00 (amount of designation fee) = 525.00 [D]

Add amounts entered at B and D and enter total at I . . . . . 980.00 [I]

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT . . . . . -0- [P]
5. TOTAL FEES PAYABLE

Add amounts entered at T, S, I, and P, and enter total in the TOTAL box . . . . . 1920.00 TOTAL

☐ The designation fee is not paid at this time.

MODE OF PAYMENT

☒ authorization to charge deposit account (see below)
☐ bank draft
☐ coupons
☒ cheque
☐ cash
☐ other *(specify)*
☐ postal money order
☐ revenue stamps

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/ US

☐ is hereby authorized to charge the total fees indicated above to my deposit account.

☒ is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

☒ is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

19-0036 — Deposit Account Number

12 March 1998 — Date *(day/month/year)*

Signature Jorge A. Goldstein

A Method for Treating or Preventing Alzheimer's Disease

Background of the Invention

Field of the Invention

The present invention is in the field of medicinal chemistry. In particular, the present invention is related to a sunrising new method to treat or prevent Alzheimer's disease by dietary restriction of carbohydrates and/or administration of an agent which causes reduction in serum insulin levels.

Related Art

According to a recent review by Mairin B. Brennan published in *Chemical and Engineering News 75(3)*:29-35 (1997), roughly 4 million people in the United States have Alzheimer's disease. Inherited or not, the disease manifests itself with progressively impaired memory leading to mental confusion as the disease systematically kills off nerve cells in the brain. (Brennan.)

The devastating consequences of Alzheimer's disease has led to a prodigious effort to identify drugs that might be useful for treating the condition. Two drugs are currently available for treating Alzheimer's symptoms. Cognex (tarcine), sold by Parke-Davis and CoCensys Inc. was approved by the FDA in 1993. Aricept, sold by Eisai of Japan, was approved late in 1996. Both drugs are designed to improve memory and cognition in the earlier stages of the disease. (Brennan.)

Alzheimer's disease is characterized by amyloid plaque that deposits around and between nerve cells in the brains. The plaques contain fibrillar aggregates of a small peptide called amyloid β-peptide. These plaques are centers for the degeneration of nerve endings. Whether the fibers themselves are themselves toxic is somewhat controversial, in view of transgenic animals which

have been engineered to express amyloid β-peptide. These mice make amyloid deposits, and there is damage to nerve cells around the plaque, however, no further neuronal loss is seen in these mice. Thus, there appear to be other mechanisms involved. (Brennan.)

Whether the amyloid plaques are the cause or the consequence of the disease is a perplexing question according to Brennan. However, "all genetic routes to Alzheimer's known today, 'act by increasing production or deposition of amyloid - or both,'" quoting Dennis J. Selkoe, professor of neurology and neuroscience at Harvard Medical School. Laedtke, *et al.*, *Clinical Research* 42(1):65A (1994), have also noted an epidemiological correlation between the deposition of amyloid in islet cells, leading to glucose intolerance and non-insulin-dependent diabetes mellitus, and amyloid β-protein deposition in brain cells, as associated with Alzheimer's disease. The authors conclude that there may be an overlap in the molecular defects that predispose to islet and brain amyloid, and therefore NIDDM and AD.

There is evidence of the over-expression of a protein called neural tread protein (NTP) in Alzheimer's disease neurons (see WO94/23756). This protein has been cloned (referred to as AD10-7), and expressed in cell-free culture.

The cathepsins are a family of enzymes that are usually located in lysosomes. It has been found that the inhibition of cathepsin D using an aspartyl protease inhibitor reduces the formation of β-amyloid protein and the resultant senile plaques. Thus inhibitors of cathepsin D, such as rhodanine derivatives, have been proposed as therapeutic agents for the treatment of Alzheimer's disease. See U.S. Patent Nos. 5,716,975 and 5,523,314.

A number of companies are seeking new therapeutic agents which cross the blood-brain barrier and inhibit amyloid deposition. One such company is Athena Neurosciences, South San Francisco, who has engineered a transgenic mouse model for the disease. Athena is sorting through hundreds of molecules in a series to look for the best pharmaceutical to take into development. (Brennan.)

One drug candidate developed by Neo-Therapeutics, Irvine, CA, is nearing clinical trials. The hypoxanthine analog (AIT-082) promotes nerve regeneration in the areas of the brain associated with memory. When the drug is administered directly to the brains of 13 month old mice, about 50% of the animals show a delay of about two months in any memory deficit and the other 50% never develop a memory deficit. This drug activates genes that express growth factor proteins known to reverse memory deficits in aged rodents when directly delivered to the brain. (Brennan.)

Another memory enhancing drug ready for clinical trials is CX516, codeveloped by Gary S. Lynch, a professor of psychobiology at the University of California, Irvine, and Gary A. Rogers, vice president of pharmaceutical discovery at Cirtex Pharmaceuticals, Irvine, CA. CX516 is an agonist of the AMPA receptor, and promotes the uptake of Ca^{2+} into nerve cells when the brain levels of glutamate are low, as they are in Alzheimer's disease. This drug reversed age-associated memory impairment in rats. (Brennan.)

An over the counter agent that may lessen the symptoms or delay the progression of the disease is the nicotine patch. According to Ken Kellar, a professor of pharmacology at the Georgetown University Medical School, Washington, D.C., epidemiological data indicate that there is a lower incidence of Alzheimer's disease among people who smoke. The nicotine patch is now being tested in 12 month clinical study. (Brennan.)

Estrogen is also being evaluated as an agent that might be helpful in protecting women from Alzheimer's disease. Preliminary results indicate that women who receive estrogen replacement therapy have a lower risk of developing the disease. (Brennan.)

Another agent being evaluated is prednisone. This drug is being tested to see if it can benefit Alzheimer's patients by reducing inflammation in their brains. A further study has just been completed which examined the antioxidant effect of vitamin E and selegiline, a drug used to treat Parkinson's disease. (Brennan.)

In completely unrelated studies, it has been reported that elevated levels of insulin in the body are responsible for many cases of obesity, diabetes, heart disease, high blood pressure, and high cholesterol levels. Michael R. Eades and Mary Dan Eades, "Protein Power," Bantam Books, New York, NY (1996). Patients with any of these conditions have been successfully treated with a dietetic regimen which is designed to reduce insulin levels, primarily by strict limitation of metabolizable carbohydrate in the diet. A further strategy is to ameliorate insulin insensitivity which progresses in severity in middle age, by adding chromium to the diet. By reducing insulin insensitivity, lower levels of insulin are required by the body to clear glucose from the blood.

Summary of the Invention

The present invention is related to the discovery that high levels of circulating insulin are a root cause of Alzheimer's disease. In particular, it has been discovered that insulin stimulates the increased expression of NTP in nerve cell culture. Since insulin crosses the blood-brain barrier, it is now clear that high levels of insulin stimulate brain nerve cells to secrete NTP and develop the hallmarks of Alzheimer's disease.

The present invention is directed to the treatment or prevention of Alzheimer's disease, in a human, comprising administering to an animal in need thereof an effective amount of an agent which results in lowered serum insulin levels. The agent useful in the present invention is one that is also useful for treating impaired glucose tolerance.

The present invention is also directed to the treatment or prevention of Alzheimer's disease, in a human, comprising restricting the metabolizable carbohydrates in the diet of the human to a level which results in lowered serum insulin levels.

The present invention also relates to a method of improving mentation of a patient with Alzheimer's disease, comprising administering to said patient an effective amount of an agent which increases the insulin sensitivity of the patient.

The present invention also relates to a method of treating or preventing Alzheimer's disease, in a human, comprising administering to an animal in need thereof an effective amount of an agent which results in lowered serum insulin levels and an agent which inhibits the formation of small strokes.

Detailed Description of the Preferred Embodiments

Animals with insulin insensitivity require higher levels of serum insulin to stimulate the metabolism of serum glucose and storage for later use. Although insulin has countless other actions in the body, the main function of insulin is to prevent serum glucose levels from rising too high. Thus, when glucose levels rise, insulin levels rise. However, when cells become resistant to insulin, the insulin receptors begin to malfunction. This malfunction appears to be a result of inherited tendencies and lifestyle abuse (over-consumption of carbohydrates). Thus, the receptors require higher levels of insulin to allow the glucose to be removed from the blood. While low levels of insulin are necessary to clear serum glucose when the insulin receptors are working optimally, insulin insensitive receptors require an excess level of insulin to keep serum glucose within the normal range.

Insulin insensitivity can be diagnosed by determining whether the animal has an elevated insulin level. In the case of humans, insulin levels of over 10 mU/ml indicate that the person has at least some insulin insensitivity. Eades and Eades, *supra*. Insulin values of 25-50 or more are very high and indicative of a high level of insulin resistance. People with insulin levels above 10 mU/ml are considered to be in need of treatment to reduce insulin levels and thereby treat, prevent or reduce the possibility of having Alzheimer's disease in the future.

Agents which may be administered to animals which lower serum insulin levels include drugs which are known to be useful for treating insulin insensitivity. One example of such an agent is chromium. The insulin receptor requires chromium to function properly. Deficiency of chromium is rampant in the American population as a diet high in starch and sugar puts a heavy demand on the insulin system to handle the incoming carbohydrates. Thus, 100-300 micrograms per day of chromium supplements may be administered, e.g. orally or systemically. Preferably, the dose is 200 micrograms of chromium per day. Preferably, the chromium is administered in the form of a chelate. A preferred chromium chelate is niacin bound chromium.

Another agent which can be used is human insulin-like growth factor I (hIGF-I). Recombinant hIGF-I has been reported to be useful for reducing hyperglycemia in patients with extreme insulin resistance. Schoenle *et al.*, *Diabetologia 34*:675-679 (1991). See also Usala *et al., N. Engl. J. Med. 327*:853-857 (1992); and Zenobi *et al., J. Clin. Invest. 89*:1908-1913 (1992). Thus, hIGF-I may be administered by intraperitoneal means to a human in need thereof to treat or prevent the onset of Alzheimer's disease. hIGF-I may be administered, e.g. systemically by injection, to the patient in need thereof in an amount effective which can be determined with no more than routine experimentation.

Other agents which can be used in the practice of the invention include dopamine agonists which have been reported to be useful for treating insulin resistance. See U.S. Patent No. 5,468,755. An example of a dopamine agonist that can be used is bromocriptine. Other dopamine agonists are described in U.S. Patent Nos. 5,597,832, 5,602,120 and 5,602,121. Thus, a dopamine agonist may be administered to a human in need thereof to treat or prevent the onset of Alzheimer's disease. Routes of administration for such dopamine agonists are described in U.S. 5,468,755, 5,597,832, 5,602,120 and 5,602,121. The dopamine agonist may be administered to the patient in need thereof in an amount effective

which is, in general, the amount required for the dopamine agonist to treat insulin resistance according to U.S. 5,468,755.

Other agents which can be used in the practice of the invention include pyruvate and pyruvate precursors which have been reported to improve insulin resistence and lower fasting insulin levels. See U.S. Patent Nos. 5,472,980 and 5,283,260.

Other agents which can be used in the practice of the invention include thiazolidinediones and related antihyperglycemic agents which have been reported to be useful for treating impaired glucose tolerance in order to prevent or delay the onset of non-insulin-dependent diabetes mellitus. See U.S. Patent No. 5,478,852. An example of a thiazolidinedione that can be used is troglitazone (brand name Rezulin™) that has recently been approved by the U.S. Food and Drug Administration for treating insulin resistance. Routes of administration for such thiazolidinediones and related antihyperglycemic agents are described in U.S. 5,478,852. The thiazolidinediones and related antihyperglycemic agents may be administered to the patient in an amount effective which is, in general, the amount effect to treat impaired glucose tolerance according to U.S. 5,478,852. See also, U.S. Patent No. 5,457,109. Unlike sulfonylureas, troglitazone is not an insulin secretagogue, "Physicians' Desk Reference," Medical Economics Company, Montvale, NJ, 2118-2119 (1998).

Additional antihyperglycemic agents include, *inter alia*, rhodanine derivatives such as the 5-methylene-2-thioxo-4-thiazolidinones, see U.S. Patent No. 5,716,975; C-substituted pentacycloazoles and N-alkyl-substituted pentacycloazoles, see U.S. Patent No. 5,641,796; hydroxyurea derivatives, see U.S. Patent Nos. 5,646,168 and 5,463,070; and piperazinylalkylpyrimidines, see U.S. Patent No. 4,980,350.

Other agents which can be used in the practice of the invention include benzothiodiazines and related antihypoglycemic agents which have been reported to be useful for treating symptomatic hypoglycemia. These agents function by suppressing insulin levels, thereby causing an increased glucose level in the blood.

An example of a benzothiadiazine which can be used is diazoxide (brand name Proglycem™) which is approved by the U.S. Food and Drug Administration for treating hypoglycemia due to hyperinsulinism. See, "Physicians' Desk Reference," Medical Economics Company, Montvale, NJ, 595-597 (1998).

A second method of the invention is directed to the treatment or prevention of Alzheimer's disease by the restriction of metabolizable carbohydrate in the diet. According to the invention, the amount of metabolizable carbohydrate is considered restricted if no more than about 55 grams are ingested per day. Preferably, no more than about 30 grams of metabolizable carbohydrates are ingested. More preferably, no more than about 15 grams of metabolizable carbohydrates are ingested. Most preferably, no more than about 10 grams of metabolizable carbohydrates are ingested. One can easily achieve these lowered levels of carbohydrate ingestion by following the regimens disclosed by Michael R. Eades and Mary Dan Eades in their book entitled "Protein Power," Bantam Books, New York, NY (1996). The regimen disclosed by Michael R. Eades and Mary Dan Eades is designed to reduce serum insulin levels to normal levels and, thereby, treat the symptoms of insulin insensitivity including obesity, diabetes, heart disease, high blood pressure and high cholesterol and triglyceride levels.

Further, one can easily adjust the levels of carbohydrates in the diet by reading nutrition labels on foods. The carbohydrate level on food labels includes the non-metabolizable fiber content. Thus, when determining the metabolizable carbohydrate amount in a serving of the food, the number of grams of fiber must be subtracted. In general, to achieve a diet which is low in metabolizable carbohydrates, one must ingest large amounts of protein from red meat, fowl and fish; vegetables including green leafy vegetables, tomatoes, peppers, avocados, broccoli, egg-plant, zucchini, green beans, asparagus, celery, cucumber, mushrooms and salads. Michael R. Eades and Mary Dan Eades disclose the amounts of metabolizable carbohydrates in a large number of foods which allows one to plan a diet that is very low in metabolizable carbohydrates. See also Robert

C. Atkins and Veronica Atkins, "Dr. Atkin's Quick and Easy New Diet Cookbook," Fireside Books, New York, NY (1997).

The present invention also relates to a method of improving mentation of a patient with Alzheimer's disease, comprising administering to said patient an effective amount of an agent which increases the insulin sensitivity of the patient. Several lines of investigation suggest a link between impaired glucose utilization and Alzheimer's disease. This hypothesis has been supported by findings that raising plasma glucose levels through glucose administration in elderly humans and rodents improves memory without affecting motor and nonmemory functions. Craft, S., *et al.,* "Effects of Hyperglycemia on Memory and Hormone Levels in Dementia of the Alzheimer Type: A Longitudinal Study," *Behav. Neurosci. 107*:926-940 (1993). Thus, according to the present invention, an agent may be administered to a patient with Alzheimer's disease to improve mentation, which agent is effective for treating insulin insensitivity. By decreasing insulin insensitivity, that is by increasing insulin sensitivity, in the patient, glucose utilization is improved in the brain and mentation will improve.

Agents which inhibit the formation of small strokes include aspirin.

The agents described herein may also be administered in conjunction with an antiinflammatory agent such as ibuprofen which has been found useful in some studies in ameliorating Alzheimer's disease.

The agents that have been described herein may also be administered with compounds which modulate ATP production and have thereby been found useful as an alternative energy source to glucose for conditions in which ischemic or hypoxic conditions have compromised ATP production. Such compounds include, *inter alia,* fructose-1,6-biphosphate, see U.S. Patent Nos. 4,546,095, 4,703,040, 4,757,052, and 5,039,665; pyruvate, see U.S. Patent No. 5,395,822; glyceraldehyde-3-phosphate and 3-phosphoglycerate, see U.S. Patent No. 5,707,971. Administration of these agents may also be useful as an alternative to insulin treatment by providing an energy source alternative to glucose, and may

obviate the general decline of aging by enhancing ATP production according to U.S. 5,707,971.

Having now generally described the invention, the same will be more readily understood through reference to the following Examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Examples

Example 1 Insulin Stimulates the Expression of AD7c-NTP, a Protein which causes neurons to exhibit neuronal sprouting and apoptosis

Insulin is an important mediator of growth and differentiation in CNS neurons. Insulin stimulated differentiation of PNET2 cells was associated with rapid (within 10 minutes) but transient increases in the levels of the 39 kD, 18 kD and 15 kD NTP species, followed by sustained increases in synthesis and steady state levels of all five NTP species. In contrast, the failure of insulin to induce differentiation of PNET1 cells was associated with absent insulin modulation of NTP.

Analysis of the signal transduction pathways demonstrated that the insulin-induced up-regulation of NTP molecules in PNET2 cells was mediated through phosphorylation of the insulin receptor substrate-1 (IRS-1) and the insulin receptor β subunit (IRβs) itself. In PNET1 cells, the lack of insulin responsiveness was associated with impaired insulin-mediated tyrosyl phosphorylation of IRS-1, but normal insulin receptor phosphorylation. Correspondingly, the insulin-stimulated association between PI3 kinase and phosphorylated IRS-1 was also impaired in PNET1 cells. In essence, impaired insulin-mediated tyrosyl phosphorylation of IRS-1 in PNET1 cells halted activation of the insulin signal transduction cascade, and subsequent events leading to modulated gene (NTP) expression. PNET1 cells lacked insulin responsiveness and failed to phosphorylate

IRS-1, but insulin receptor levels and tyrosyl phosphorylation (PY) of the β-subunit were intact. PNET2 cells responded to insulin stimulation with phosphorylation of IRS-1, up-regulation of NTP, and neuronal differentiation. The results were confirmed by absent association between PI3 kinase and IRS-1-PY in PNET1 cells after insulin stimulation.

Neuritic sprouting and neuronal differentiation were induced in PNET2 and SH-Sy5y cells by insulin, PMA, or RA stimulation. Insulin-mediated neuritic growth was associated with increased expression of the fetal brain and PNET-dominant forms of NTP (15 kD and 18 kD). In contrast, the PMA- and RA-induced neuritic sprouting modulated expression of the 21 kD and 26 kD NTP species, which are primarily expressed in the mature brain, and accumulated in AD brains. Thus, expression of the immature or fetal forms of NTP are regulated by mechanisms and growth factors distinct from those involved in modulating expression of the 21 kD and 26 kD NTP molecules. Therefore, expression of fetal NTP molecules/genes can be mediated through the IRS-1 cascade, whereas expression of adult brain/AD-associated NTP genes can be regulated mainly through protein kinase C pathways.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions without undue experimentation. All patents, patent applications and publications cited herein are incorporated by reference in their entirety.

What Is Claimed Is:

1. A method for the treatment or prevention of Alzheimer's disease, in a human, comprising administering to a human in need thereof an effective amount of an agent which results in lowered serum insulin levels.

2. The method of claim 1, wherein said agent is chromium.

3. The method of claim 2, wherein said chromium is ingested orally by said human in an amount of from about 100 to 300 micrograms per day.

4. The method of claim 2, wherein said chromium is administered in the form of a chelate.

5. The method of claim 4, wherein said chromium chelate is niacin bound chromium.

6. The method of claim 1, wherein said agent is insulin-like growth factor.

7. The method of claim 1, wherein said agent is a dopamine agonist.

8. The method of claim 7, wherein said dopamine agonist is bromocryptine.

9. The method of claim 1, wherein said agent is a thiazolidinedione.

10. The method of claim 9, wherein said thiazolidinedione is troglitazone.

11. A method for the treatment or prevention of Alzheimer's disease, in a human, comprising restricting the metabolizable carbohydrates in the diet of the human to a level which results in lowered serum insulin levels.

12. The method of claim 11, wherein the metabolizable carbohydrates in the diet are limited to no more than about 55 grams per day.

13. The method of claim 11, wherein the metabolizable carbohydrates in the diet are limited to no more than about 30 grams per day.

14. The method of claim 11, wherein the metabolizable carbohydrates in the diet are limited to no more than about 15 grams per day.

15. The method of claim 11, wherein the metabolizable carbohydrates in the diet are limited to no more than about 10 grams per day.

16. A method for the treatment or prevention of Alzheimer's disease, in a human, comprising administering to a human in need thereof an effective amount of an agent which results in lowered serum insulin levels and restricting the metabolizable carbohydrates in the diet of the human.

17. The method of claim 16, wherein said agent is selected from the group consisting of chromium, insulin-like growth factor, a dopamine agonist and a thiazolidinedione.

18. The method of claim 16, wherein said agent is troglitazone.

19. The method of claim 16, wherein the metabolizable carbohydrates in the diet are limited to no more than about 55 grams per day.

20. The method of claim 16, wherein the metabolizable carbohydrates in the diet are limited to no more than about 30 grams per day.

21. The method of claim 16, wherein the metabolizable carbohydrates in the diet are limited to no more than about 15 grams per day.

22. The method of claim 16, wherein the metabolizable carbohydrates in the diet are limited to no more than about 10 grams per day.

23. A method of improving mentation of a patient with Alzheimer's disease, comprising administering to said patient an effective amount of an agent which increases the insulin sensitivity of the patient.

A Method for Treating or Preventing Alzheimer's Disease

Abstract

Disclosed is a method for treating or preventing Alzheimer's disease by restricting the level of metabolizable carbohydrate in the diet and/or administering to the patient an effective amount of an agent which reduces serum insulin levels.